FOSSIL BONE RECOVERY FROM SEDIMENT RESIDUES BY THE 'INTERFACIAL METHOD'

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ABSTRACT. New methods for the separation of microvertebrate fossils from particulate sediment residues are based on preferential wetting of the bone particles by water-immiscible solvents in aqueous media. Three embodiments of this basic idea are described, two of them involving adhesion of the bone particles to solid or gelatinous materials (polystyrene or high molecular weight hydrocarbons), whilst the third relies on simple two-phase liquid mixtures. The new methods give results which compare favourably with those from conventional techniques of bone-enrichment, and at minimal or negligible cost. In small-scale experiments, efficient separation of bone has been achieved from gangue minerals consisting largely of quartz, limonite, calcite, siltstone, and claystone, and of particle sizes ranging between 1·4 mm and 0·35 mm. Multi-stage separations are possible, allowing one to obtain high-purity concentrates virtually quantitatively from sediment residues of low initial bone content. One such exercise is demonstrated on the large-scale, using 1 kg of quartz-rich sediment residues.

The use of a new reagent, sodium hydrogenoxalate, for the digestion of limonitic gangues, is briefly described, as are improvements relating to conventional bromoform density separations.

IN recent years microvertebrate fossils have attracted increased attention from palaeontologists, due in large part to a growing interest in the origins of the major vertebrate groups, the early representatives of which tended to be small animals. Also, as small vertebrates are often important ecologically, and as they frequently consist of the young of larger animals, their fossil remains would be expected to yield data important in the reconstruction of ancient environments and animal communities. Also, microvertebrates can be useful in stratigraphy.

The recovery of microvertebrate fossils from a sediment typically involves a three-stage process, requiring the palaeontologist (1) to disaggregate the sediment to give a particulate residue of the relevant size-range, from which (2) unwanted gangue minerals are removed by a variety of processes to give a bone-enriched concentrate, which (3) is hand-picked for specimens of interest. Unfortunately, the bone-enrichment processes hitherto available for stage 2 are usually not very cost-effective, and the yield of microvertebrates from a sediment is often limited by the then inevitably tedious nature of stage 3.

Bone-enrichment processes fall roughly into two categories, depending on whether the gangue minerals are removed chemically or physically. Chemical methods include the dissolution of calcareous gangues with acetic or formic acid solutions (see Rixon 1976) and of limonitic gangues using thioglycollic acid (Freeman 1979, following Howie 1974). Physical methods include 'jigging' on a sieve in water (for lignite—Kühne 1968), flotation in either chlorinated hydrocarbons (for lignite—Freeman 1975) or brominated hydrocarbons (usually for quartz—see, for example, Griffith 1954), and use of a photoelectric scanner to separate bone and gangue particles by means of their colour differences (Kühne 1970). All these techniques have one or more drawbacks, usually limited applicability, low efficiency, high cost, and reagent hazard.

Whilst engaged in a search for mammal teeth in a Lower Cretaceous bone bed, I have developed a suite of remarkable new procedures which allow bone-bearing sediment residues to be enriched with higher efficiency and/or much lower cost than any other method previously described. As these procedures exploit phenomena at the interfaces of mutually immiscible liquids, I have named them collectively the 'Interfacial Method'.

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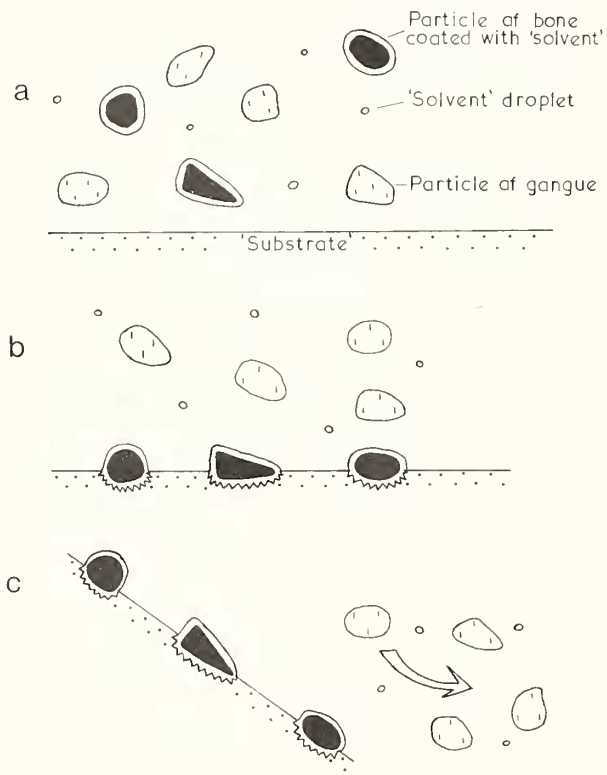
THE INTERFACIAL METHOD—GENERAL DESCRIPTION

The new procedures are all based on a seemingly unknown property of fossil bone, namely that when contacted with a two-phase mixture of water and a water-insoluble organic liquid, it is wetted by the organic liquid more readily than are the gangue minerals. Three general procedures, differing only in the way that this phenomenon is exploited, have been investigated experimentally, and are described herein.

In two of these, called Procedures A and B, the particulate bone-bearing residue is gently agitated with a suspension of the water-insoluble organic liquid (hereafter for brevity called the 'solvent') in a dilute aqueous detergent solution, whereupon the particles of bone become preferentially coated with the 'solvent'. The mixture is then briefly brought into contact with a 'substrate', a solid or gelatinous material whose composition is such that it softens in contact with the 'solvent' (see text-fig. 1*a*). When the bone particles make contact with such a 'substrate', they become attached to it by means of their 'solvent' coatings (see text-fig. 1*b*). Subsequent removal of the unattached material leaves a concentrate enriched in bone particles adhering to the surface of the 'substrate' (see text-fig. 1*c*).

In more detail, the 'substrate' of Procedure A consists of polystyrene, a low-cost polymer widely used in food-packagings; the 'solvent' can be one of a number of suitable aromatic or chlorocarbon solvents, but is preferably tetrachloroethylene, a relatively inexpensive dry-cleaning agent. The concentrate adhering to the polystyrene is removed either by gentle brushing (after rinsing with light petroleum spirit and air-drying), or if it is too firmly attached, by dissolving the polymer in a suitable solvent. Alternatively, the concentrate may be searched *in situ* for specimens of interest.

In Procedure B, the solvent is even cheaper, being domestic paraffin (kerosene), while the 'substrate' is a high molecular weight solid or gelatinous hydrocarbon such as either petroleum



TEXT-FIG. 1. The mechanism of the Interfacial Method, Procedures A and B. In aqueous suspension, particles of fossil bone are selectively coated with water-insoluble organic solvent (*a*), which allows them to adhere to a suitable 'substrate' (*b*). Removal of the unattached gangue minerals (*c*) leaves a concentrate of bone particles on the surface of the 'substrate'.

jelly (which is coated on to a suitable, inert support) or paraffin wax. The concentrates from Procedure B are recovered by melting the 'substrate' materials in hot water containing a little detergent. This also allows the 'substrate' materials to be recovered for reuse, at a considerable saving in cost.

In the third procedure described in this paper, called Procedure C, the sediment residue is briefly agitated with a simple mixture of tap water (*not* containing detergent) and an immiscible organic liquid (in practice, domestic paraffin), which is then allowed to settle momentarily. The interface between the two liquids holds in suspension a small quantity of material enriched in bone, which is recovered merely by decanting the liquids through a fine wire mesh.

As to their effectiveness, Procedures A and B have given concentrates which may be hand-picked without the need of further enrichment. This is not true for Procedure C, where the degree of enrichment achieved so far, although potentially useful, has not been spectacularly high. However, its fundamental simplicity and the low cost of its materials make Procedure C an attractive prospect for large-scale mechanized sediment processing.

Procedures A, B, and C are described in more detail in the 'Experimental' section (following).

EXPERIMENTAL

The following account describes firstly a limited investigation on the small-scale of some of the more obvious variables relating to the new procedures, the information from which is then used in larger-scale experiments more appropriate to the practical needs of the palaeontologist.

The overall performance of an experiment was measured by three parameters, the relative percentages of bone in the starting material and in the resulting concentrate, and the percentage of the bone originally present in the test sample that was recovered in the concentrate (called hereafter the 'recovery of bone'). The percentage compositions of the starting materials and the products were estimated by hand-sorting representative samples, usually under natural daylight and using low-power magnification. Under such conditions, identification of the bone particles did not present much of a problem, except when the gangue was lignite (Experiment 8). Unless indicated to the contrary, for the small-scale experiments the percentage compositions and bone-recoveries are based on the numbers of particles (rather than on their weights); the exceptions are where the gangue particles were fragile and liable to disintegration during the experiment.

In the tables, to facilitate comparison the starting material weights have been standardized to 5·00 g or 25·00 g; similarly the term 'concentrate' covers *all* residues which either adhere to a 'substrate' (for Procedure A) or are carried over by decantation (for Procedure C), irrespective of whether any enrichment of bone has in fact occurred (see, for example, Experiment 33).

Finally, for simplicity the term 'bone' is used in a loose sense to cover all phosphatic fossils, including teeth and coprolites.

THE ORIGIN OF THE EXPERIMENTAL MATERIALS

To make the experiments as realistic as possible, actual residues obtained from sediment processing were used in preference to 'tailor-made' mineral mixtures, which in some ways would have made for more 'elegant' experiments. Emphasis was placed on quartz-rich residues of 0·5–1·0 mm particle size, largely because such materials are especially laborious to search manually and yet often contain specimens of interest.

Details of the procedures used to break down the sediments are given, as these, by affecting the surface properties of the resulting particulate residues, might have had an influence on the experimental results.

?Telham Bone Bed. This sediment is a thin pebbly ferruginous sandstone, found attached to the upper surfaces of massive blocks of fine-grained sandstone fallen from East Cliff, Hastings (map reference TQ 832096). It is suspected to be the Telham Bone Bed from the descriptions given in Allen 1949, pp. 279–282 and Allen 1962, pp. 224–225. If so, it is part of the Wadhurst Clay Formation, and probably is of Valanginian age (Lower Cretaceous) (see Clemens and Lees 1971, pp. 120–121). So far, 12·5 kg of the sediment has yielded at least four mammal teeth, as yet undescribed, as well as remains of fish (*Lepidotes*, *Hybodus*, *Lonchidion*, and *Hylaeobatis*), turtles, crocodiles (goniopholids and *Bernissartia*), and dinosaurs (*Iguanodon* and *Megalosaurus*).

After digestion with dilute acetic and formic acid solutions, the particles in the resulting residues were still heavily coated and partially aggregated by a tenaceous limonitic cement. As the Interfacial Method exploits the surface properties of the fossil bone particles, obviously they must not be attached to, or coated with, substantial amounts of such gangue minerals. The limonitic cement was removed from the 0·5–1·0 mm fraction by digestion in 10·0 g batches with 150 ml portions of concentrated sodium hydrogenoxalate solution at *c.* 100 °C for an hour. The oxalate solution was prepared by the partial neutralization of oxalic acid (10·0 g of the anhydrous acid) with sodium bicarbonate (9·3 g) in 150 ml of calcium-free water. Its reactivity towards limonite represents the satisfactory compromise between oxalic acid solution, which dissolves limonite but also attacks fossil bone, and sodium oxalate solution, which is inert. As a reagent for limonite, sodium hydrogenoxalate offers considerable advantages of cost and general convenience over thioglycollic acid (see Howie 1974 and Freeman 1979).

After the oxalate digestion, the 0·5–1·0 mm material consisted of a mixture of clean and separate quartz, limonite, and bone particles. It was used in most of the small-scale experiments (nos. 1, 2, 9–33), either as such, or after further separation by conventional bromoform flotation and/or the Interfacial Method.

Cliff End Bone Bed. This material, of similar age to the Telham Bone Bed, occurs as loose blocks of coarse ripple-marked sandstone at Cliff End, Kent. It too contains mammal teeth, which in recent years have been collected by systematic processing involving acid-digestion and heavy-liquid density separation (see Clemens 1963, Kermack, Lees and Mussett 1965, and Clemens and Lees, 1971). Kermack *et al.* mentioned the recovery of nine mammal teeth from 5 cwt. (254 kg) of matrix using such methods.

The material used for the scaled-up Interfacial Method separations (Experiments 34–9) was part of that yielded by digestion of 58 kg of the Cliff End Bone Bed with dilute formic and acetic acid solutions. It consisted largely of separate, clean particles of quartz and fossil bone, and thus did not require any after-treatment, except for thorough washing with water and detergent to remove fine dust.

Kirtlington Mammal Bed. This is a thin bed of mammaliferous marl within the Forest Marble (Middle Jurassic) of the Old Cement Works Quarry, Kirtlington, Oxfordshire (see Freeman 1979, and Ware and Whatley 1980).

The material used for Experiment 4 was produced by wet-sieving of the sediment, and consisted largely of a mixture of comminuted shell fragments and ooliths. Experiment 3 used the insoluble limonitic residue from acetic acid digestion of such a washing residue.

'*Cowleaze Chine Pellet Bed*'. This is an undescribed channel deposit at the base of the Wealden Shales (Lower Cretaceous), near the mouth of Cowleaze Chine, Isle of Wight (map reference SZ 443801). It consists of a small lenticular mass of clay pellets in a calcareous siltstone matrix, and was disaggregated using dilute acetic acid solution to give the material used for Experiment 5. The vertebrate fauna consists principally of elasmobranchs, and was itemized in Freeman 1975 (Table 1, under 'Undescribed channel deposit').

Perna Bed. This is a glauconitic sandstone, rich in marine molluscs and corals, and locally, in fish bones and teeth, at the base of the Lower Greensand (Aptian, Lower Cretaceous) in the Isle of Wight. The particular sample used in Experiment 6 came from Atherfield Point—see Osborne White 1921, p. 24, and was disaggregated using dilute acetic acid solution.

Cyrena Limestone. This is a thin-bedded silty limestone crowded with freshwater lamellibranchs, occurring repeatedly within the Wealden Shales (Lower Cretaceous) of the Isle of Wight (see Osborne White 1921, p. 15). The particular sample used for Experiment 7 was found loose on the beach south-east of Shepherd's Chine (map reference SZ 448796), and was broken down with dilute acetic acid solution.

'*Headon Hill Lignite Bed*'. This is an irregular seam of friable, mammaliferous lignite in the Upper Headon Beds (Upper Eocene) of Headon Hill, in the Isle of Wight (see Cray 1973, pp. 24–27). The material used for Experiment 8 was obtained by wet-sieving the sediment.

PROCEDURE A

A typical small-scale experiment (*Experiment 1*)

A 5·00 g sample of 0·5–1·0 mm residue from the ?Telham Bone Bed, containing 13% bone, was saturated with tetrachloroethylene (2·6 g, 1·6 ml), and agitated briefly in a 250 ml 'Pyrex' beaker with 50 ml of a 1% solution of a proprietary 'washing-up liquid' ('Fairy Liquid', ex Procter and Gamble Ltd.) in cold tap-water. The mixture was then poured rapidly from the beaker into a

160 ml container made of unfoamed polystyrene (an empty yogurt carton), where it was kept for *c.* 15 seconds, before being transferred as completely as possible to another such container. The surfaces of the polystyrene that had come into contact with the mixture had been somewhat corroded and softened by the tetrachloroethylene, and to them adhered a concentrate consisting predominantly of fossil bone particles. The process was repeated another five times, the yield and purity of the concentrates attached to the polystyrene decreasing steadily in amount and purity from the first container to the sixth. In total, the concentrates weighed 0·43 g, contained 78% of bone and constituted a bone recovery of 85%. The material that did not become attached to the polystyrene (the tailings) weighed 4·57 g, and contained 2·1% of bone.

The nature of the sediment residue and 'solvent' type in Procedure A separations (Experiments 1–21)

Using the above method, various aspects of Procedure A have been investigated experimentally. Enrichment in bone has been achieved for sediment residues consisting largely of quartz, limonite, calcite (ooliths and shell fragments), claystone, and siltstone (see Table 1, Experiments 1–6), of particle sizes ranging between at least 0·35 mm and 1·4 mm (Experiments 9 and 10), and containing as much as 61% of bone (Experiment 11) and as little as 0·8% (Experiment 12). Only the separation

TABLE 1. The scope of the Interfacial Method, Procedure A. Abbreviations: ?TBB = ?Telham Bone Bed; KMB = Kirtlington Mammal Bed; CCPB = 'Cowleaze Chine Pellet Bed'; PB = Perna Bed; CL = Cyrena Limestone; HHLB = 'Headon Hill Lignite Bed'; C_2Cl_4 = tetrachloroethylene. All the percentages quoted for Experiments 3, 7, and 9 are based on the weights of the particles, and not on their numbers. The experimental procedures were as described for Experiment 1.

	STARTING MATERIAL (5·00g)				SOLVENT	TAILINGS		CONCENTRATE		
Expt. no.	Source	Chief gangue mineral(s)	Particle size	% bone	Volume of C_2Cl_4	Wt.	% bone	Wt.	% bone	Recovery of bone
1	?TBB	99% quartz	0·5–1·0 mm	13%	1·6 ml	4·57g	2·1%	0·43g	78%	85%
2	?TBB	95% limonite	0·5–1·0 mm	9·1%	1·6 ml	4·69g	3·5%	0·31g	91%	41%
3	KMB	97% limonite	0·5–1·0 mm	31%	4·0 ml	3·86g	23%	1·14g	52%	39%
4	KMB	88% calcite	0·5–1·0 mm	1·0%	1·5 ml	4·89g	0·8%	0·11g	6·2%	18%
5	CCPB	89% claystone	0·5–1·0 mm	1·3%	1·6 ml	3·87g		1·13g	6·2%	42%
6	PB	37% siltstone, 62% quartz	0·5–1·0 mm	4·3%	1·5 ml	4·72g	2·8%	0·28g	21%	33%
7	CL	c.100% pyrites	0·5–1·0 mm	19%	1·2 ml	4·38g	16%	0·62g	31%	20%
8	HHLB	94% lignite	0·5–1·0 mm	2·8%	0·8 ml	4·21g		0·79g	3·8%	28%
9	?TBB	limonite>quartz	1·0–1·4 mm	7·3%	3·1 ml	3·78g	1·7%	1·22g	25%	83%
10	?TBB	51% quartz, 49% limonite	0·35–0·5 mm	12%	1·6 ml	4·83g	8·9%	0·17g	83%	43%
11	?TBB	limonite	0·5–1·0 mm	61%	1·6 ml	1·12g	9·6%	3·88g	83%	97%
12	?TBB	quartz	0·5–1·0 mm	0·8%	1·8 ml	4·72g	0·5%	0·28g	5·5%	57%

TABLE 2. The results of two three-stage Procedure A separations. The experiments are continuations of Experiment 1 (*top*) and Experiment 2 (*bottom*), the starting materials for stages 2 and 3 being the tailings from stages 1 and 2 respectively.

STARTING MATERIAL			STAGE 1	STAGE 2	STAGE 3	RECOVERY OF BONE
?Telham Bone Bed, 0·5–1·0 mm., 13% bone, 86% quartz.	% bone in	concentrate	78	52	5·5	85+8+4 = 97%
		tailings	2·1	0·8	0·5	
?Telham Bone Bed, 0·5–1·0 mm., 9·1% bone, 86·0% limonite, 4·9% quartz.	% bone in	concentrate	91	23	1·5	41+49+8 = 98%
		tailings	3·5	0·9	0·5	

of bone from pyrites and lignite (Experiments 7 and 8) has proved difficult, apparently because these gangue minerals are nearly as readily wetted by the tetrachloroethylene 'solvent' as is the bone.

Multiple-stage extractions are possible (see Table 2), permitting the exhaustive extraction of a sediment for its microvertebrate content. As would be expected, the efficiency of a particular extraction is dependent upon the concentration of bone in the material being processed, and so a progressive decline occurs in the purity of the concentrates from stages 1 to 3.

A variety of chlorocarbon and aromatic 'solvents' have been used in Procedure A (see Table 3), of which the most successful was tetrachloroethylene. *Under the particular conditions prevailing*

TABLE 3. The variation of the nature of the 'solvent' in Procedure A separations. The experimental procedures were as described for Experiment 1.

STARTING MATERIAL:- 5·00g of ?Telham Bone Bed, 0·5–1·0mm fraction containing 13% bone and 86% quartz

Expt. no.	SOLVENT	TAILINGS Wt.	TAILINGS % bone	CONCENTRATE Wt.	CONCENTRATE % bone	CONCENTRATE Recovery of bone
1	1·6ml tetrachloroethylene	4·57g	2·1%	0·43g	78%	85%
13	1·8ml carbon tetrachloride	4·37g	3·1%	0·63g	40%	65%
14	1·1ml dichloromethane	4·74g	6·0%	0·26g	58%	44%
15	1·4ml chloroform	4·83g	7·7%	0·17g	70%	38%
16	1·4ml 1,2-dichloroethane	4·79g	8·9%	0·21g	83%	39%
17	1·7ml bromoform	3·05g	1·1%	1·95g	16%	92%
18	1·3ml xylene	4·44g	4·9%	0·56g	59%	80%
19	1·6ml toluene	4·72g	6·3%	0·28g	92%	58%
20	1·4ml benzene	4·96g	12%	0·04g	55%	6%
21	1·5ml paraffin	5·00g	13%	0·00g		0%

during an experiment the 'solvent' must have a positive, but only moderate, ability to attack the polystyrene 'substrate'. Consequently, Experiment 21, using paraffin, a non-solvent for polystyrene, failed because no material became attached to the 'substrate', while at the other extreme, when bromoform, an excellent solvent for polystyrene, was used, the polystyrene became coated with too much material, most of it gangue, and containing only slightly more bone than the starting material (Experiment 17). Presumably, a 'solvent' with optimum properties could be prepared by the judicious blending of two or more different solvents.

The quantity of the 'solvent'

All other factors being kept the same, one would expect the performance of a Procedure A separation to depend, not merely on the nature of the 'solvent' used, but also upon its quantity.

In the only case where this has been systematically studied (using the same, quartz-rich, experimental material as Experiment 23 (q.v.) and the method of Experiment 1), as the tetrachloroethylene was increased in amount from 1·5 ml to 2·0 ml, an increase in percentage recovery (from 28 to 42%) and a decrease in concentrate purity (from 85 to 69%) occurred. Curiously, these trends were not continued when the 'solvent' volume was further increased, eventually by sevenfold to 14 ml, the percentage recovery and concentrate purity remaining essentially static at 42 and 73% respectively.

In contrast, a few, limited experiments suggest that when the gangue mineral is limonite, pyrites, or lignite, increasing the quantity of 'solvent' produces dramatic increases in percentage recovery but with corresponding decreases in concentrate purity.

The necessity of a detergent in Procedure A (Experiment 22)

When detergent was omitted from a repeat of Experiment 1, the tetrachloroethylene remained as a discrete layer and did not disperse into fine droplets as before. Only a single polystyrene container was used, and to this became attached 0·99 g of material containing 33% of bone (cf. 78% in Experiment 1 and 13% in the starting material). So while a detergent greatly increases the efficiency of Procedure A, it seems not to be essential to it. The reverse is expected to be true for Procedure C.

Is there a 'palaeontological bias' in the Interfacial Method?

The Interfacial Method exploits subtle differences between the surface properties of the bone and gangue particles. It is even possible that differences in shape and texture between the bone particles themselves could be of significance. One would expect those bone particles having a relatively high surface area, such as flat fish scales and splinters of cancellous bone, to be the ones most readily wetted by the 'solvent', and therefore to be slightly more frequently recovered than would the relatively compact teeth of fish and reptiles. If so, in a multiple stage extraction, the proportion of the latter should progressively increase with each stage of the extraction. Interestingly, such an increase *has* been seen in one of the three-stage extractions reported earlier (see Table 4), and so this kind of 'palaeontological bias' should be borne in mind when using the Interfacial Method, although it is unlikely to be of major practical importance.

TABLE 4. 'Palaeontological bias' in a three-stage Procedure A separation. Note the progressive increase in the percentage of teeth (of *Lepidotes*, *Caturus*, *Lonchidion*, *Hybodus*, and crocodiles) in the concentrates from the three stages. The experiment is the continuation of Experiment 2 reported in Table 2, *bottom*.

STARTING MATERIAL:– ?Telham Bone Bed, 0·5–1·0mm fraction, containing 9·1% bone, 86·0% limonite and 4·9% quartz

	STAGE 1	STAGE 2	STAGE 3	TAILINGS
Number of teeth	19	87	25	6
Total no. of bone particles	721	860	132	34
Percentage of teeth	2·6%	10·1%	18·9%	17·6%

PROCEDURE B

A typical small-scale experiment (*Experiment 23*)

A 25·00 g sample of 0·5–1·0 mm residue from the ?Telham Bone Bed, containing 14% bone, 67% quartz, 16% limonite, and 3% lignite, was saturated with domestic paraffin (7·1 g, 9·2 ml) and washed into a 900 ml screw-cap bottle with 250 ml of a 1% solution of a proprietary washing-up liquid ('Fairy Liquid') in cold tap-water. A plastic cup, whose dimensions were such that it could be fitted snugly into the neck of the screw-cap bottle, had its base removed with a hot knife; it was then smeared heavily inside and out with petroleum jelly and inserted in the neck of the bottle. The bottle was then sealed, held horizontally, and agitated for 1 minute so that its contents played over the surface of the plastic cup. A small amount of a bone-rich concentrate remained stuck to the petroleum jelly on the cup, which was then rinsed in cold water to remove non-adherent material rich in gangue. This rinsing step is essential to maintain the richness of the concentrate—its omission in an otherwise identical experiment led to a reduction in the bone content of the concentrates (from the first ten extractions) from 56 to 27%.

In all, forty such extractions were performed, using a freshly prepared cup for each extraction. The plastic cups were then briefly immersed one by one in hot water containing a little detergent, whereupon the petroleum jelly melted and floated to the surface to form a curd, which was recovered for reuse. The bone-rich concentrates thus released accumulated in the bottom of the hot water bath.

Results. Concentrate from extractions 1–10: 3·45 g, 56% bone, recovery of bone = 76%.
Concentrate from extractions 11–40: 2·45 g, 14% bone, recovery of bone = 15%.
Tailings: 19·10 g, 1·2% bone.

Comparison of Procedure B with a density separation using bromoform

A fresh 25·00 g sample of the same material as that used for Experiment 23 was used in an essentially conventional heavy-liquid density separation (Experiment 24). Initially, this used neat bromoform (stabilized with ethanol, specific gravity 2·63–2·69), and gave a heavy fraction containing 20% of bone and weighing 4·13 g. A little ethanol was then added to lower the specific gravity to *c.* 2·57, and a second heavy fraction was obtained, this time containing 27% of bone and weighing 3·40 g. The procedure was, to some extent, optimized by being carried out in a 50 ml measuring cylinder, whose height : diameter ratio was more favourable to efficient use of the expensive flotation liquid and to clean separation of the heavy and light fractions than the beakers used by Kermack *et al.* (1965). In spite of this, separation of the bone was inefficient, as can be seen from Table 5, due to its wide density range, which overlapped those of the gangue minerals limonite and quartz.

Clemens and Lees (1971) describe some procedures for removing brominated hydrocarbons from flotation residues. These include washing in 70% alcohol in a fume cupboard, and drying in a vacuum oven evacuated through two vapour-traps cooled with liquid nitrogen. Whilst it is essential to remove all traces of brominated hydrocarbons from sediment residues prior to hand-picking because of their high toxicities (see, for example, bromoform in Sax 1975, p. 476), the use of a vacuum oven, etc., is unwieldy and expensive.

A much simpler procedure was developed to clean the residues from Experiment 24. Firstly, they were washed with methylated spirit and then water. They were then suspended in a fine wire mesh over a can of boiling water heated *out of doors*; this steaming procedure was continued until the steam issuing from the residues no longer smelt of bromoform. The procedure employs the technique of steam distillation (see Vogel 1956, p. 12) whereby water-insoluble organic compounds of high boiling-point can be vaporized at relatively low temperatures by admixture with steam. It was during one such bromoform-removal exercise in April 1979 that the Interfacial Method itself was discovered; ironically this will probably render the bromoform-density separation method largely obsolete, and with it the innovations reported in this section.

As a final precaution, the steamed residues were air-dried outdoors in strong sunlight, which should have served to oxidize photochemically any last traces of bromoform; alternatively, these could have been hydrolysed by treating the residues with a hot, dilute alkali solution.

The results of Experiment 24 are summarized in Table 5, and compared with those of the Procedure B separation described earlier. The overwhelming superiority of Procedure B is obvious from these results. Particular attention should be paid to the relative costs of the two methods and to the fact that Procedure B uses freely available materials which are virtually free of hazard. The only possible disadvantage of Procedure B compared to bromoform density separation is the relatively more energetic conditions it employs, which might increase the risk of damage to the fossils. In practice, I do not consider that this is a significant problem, as the particles of bone are initially cushioned against damage by the oily droplets enveloping them, and soon afterwards become embedded in the 'substrate'. Furthermore, the fossils isolated by Procedure B do not seem to be noticeably more fragmented than their counterparts isolated in other ways. In any event, the vastly increased quantity of sediment that can now be processed by Procedure B more than compensates for any specimens lost through breakage. This is true also for Procedures A and C, which employ less energetic conditions than Procedure B.

TABLE 5. A comparison of the performances of the Interfacial Method (Procedure B) and a conventional bromoform density separation. The costs are calculated from U.K. prices current at the time of the experiments (August 1979).

STARTING MATERIAL:– 25·00g of ?Telham Bone Bed, 0·5–1·0mm fraction, containing 14% bone, 67% quartz, 16% limonite and 3% lignite

	TAILINGS		CONCENTRATE			REAGENTS and COST
	Wt.	% bone	Wt.	% bone	Recovery of bone	
Interfacial Method, Procedure B, Expt.no. 23, extractions 1–10	21·55g	2·7%	3·45g	56%	76%	24g petroleum jelly £0·03 9·2ml paraffin £0·001
Conventional bromoform density separation (Expt. no. 24)	17·47g	7·0%	7·53g	23%	59%	18ml bromoform £1·18 0·8ml ethanol £0·0003

PROCEDURE C

A typical small-scale experiment (Experiment 25)

A 5·00 g sample of 0·5–1·0 mm residue from the ?Telham Bone Bed, containing 7·5% of bone and 91·5% of quartz, was placed in a 250 ml 'Pyrex' beaker, to which was added 25 ml of domestic paraffin and 25 ml of cold tap-water. Almost immediately thereafter, the water/paraffin mixture was decanted through a fine wire mesh (a tea-strainer), care being taken to ensure that the sediment residue at the bottom of the beaker did not slop over into the wire mesh. The interface between the two liquids held in suspension a small quantity of material enriched in bone, which was filtered off by the wire mesh. The water/paraffin mixture was returned to the beaker and the extraction repeated, in all for a total of 100 times over 15 minutes. In these extractions, vigorous agitation of the beaker and its contents tended to be counter-productive, adequate mixing being achieved simply by the return of the liquids to the beaker. A bone-enriched concentrate (0·77 g, 21% bone) accumulated in the wire mesh, leaving the tailings (4·23 g, 2·5% bone) in the beaker.

The effect of container geometry and solvent type upon Procedure C separations (Experiments 25–33)

Only two variables that might possibly have an effect upon Procedure C have so far been investigated, and both produced unexpected results. It was expected that the geometry of the container and of the liquids within it would be important factors in Procedure C separations. More specifically, the total area of the paraffin/water interface, and its vertical distance above the sediment residue should have a marked influence on the purity of the concentrate and on the percentage recovery of bone. However, contrary to such expectations, Experiments 26 and 27 both gave similar results to Experiment 25 (see Table 6), even though the interface areas and vertical distances ranged from 12 cm² and *c.* 22 mm for Experiment 26 to 87 cm² and *c.* 7 mm for Experiment 27.

TABLE 6. An investigation of container geometry and solvent type in Interfacial Method (Procedure C) separations. The experimental procedures were as described for Experiment 25 (except for the 'variable').

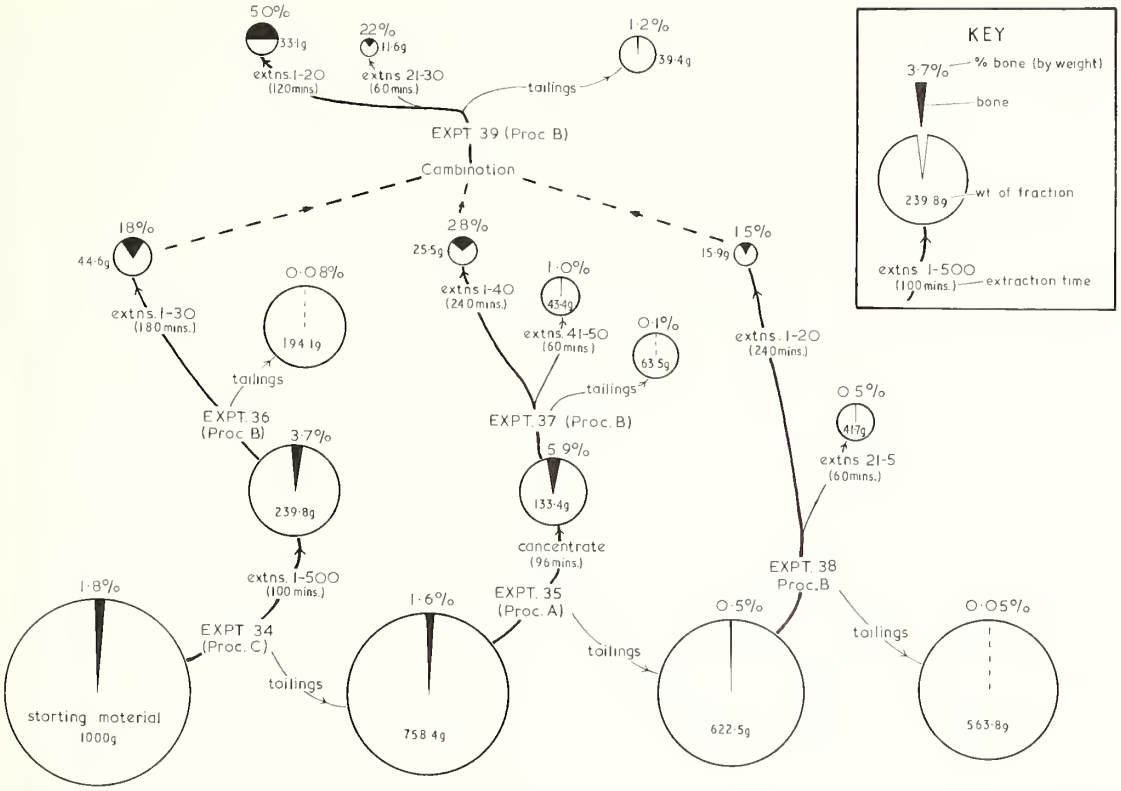
STARTING MATERIAL:– 5·00g of ?Telham Bone Bed, 0·5–1·0mm fraction containing 7·5% bone and 91·5% quartz

	VARIABLE	TAILINGS		CONCENTRATE		
Expt. no.		Wt.	% bone	Wt.	% bone	Recovery of bone
	CONTAINER:–					
26	50ml beaker	4·57g	2·3%	0·43g	16%	48%
25	250ml beaker	4·23g	2·5%	0·77g	21%	70%
27	1 litre beaker	4·37g	2·1%	0·63g	13%	59%
	SOLVENT:–					
28	toluene	3·41g	3·2%	1·59g	8·5%	63%
29	xylene	3·48g	6·1%	1·52g	7·9%	40%
30	tetrachloroethylene	0·08g	3·3%	4·92g	7·5%	99%
31	chloroform	0·00g		5·00g	7·5%	100%
32	petrol	0·00g		5·00g	7·5%	100%
33	corn oil	0·00g		5·00g	7·5%	100%

Experiment 25 was also repeated with the paraffin being replaced by equal volumes of other organic liquids of widely varying physical properties, notably viscosity and density. Surprisingly, with the doubtful exceptions of toluene and xylene, none of these other liquids separated bone from the gangue particles (see Experiments 28–33).

SCALED-UP 'INTERFACIAL METHOD' SEPARATIONS

The techniques used in the small-scale experiments described earlier have been modified to make them more applicable to a scale large enough to be of practical use to the palaeontologist. I can see no reason why these scaled-up variants could not themselves be further scaled-up as desired, and without limit.



TEXT-FIG. 2. A large-scale separation of bone from quartz-rich sediment residue using the Interfacial Method. The starting material represented the 0·7–0·85 mm residue from 31·9 kg of the Cliff End Bone Bed. The areas of each circle and of the black sector within it are proportional to the weights of the fraction concerned, and of the bone therein, respectively. Abbreviations: Expt., Experiment; extns., extractions; Proc., Procedure; mins., minutes. For details of Experiments 34, 35, and 38, please see the text.

To sum up, in the experiments described in this section 1000 g of quartz-rich sediment residue, containing 1·8% by weight of bone, was separated into 33·1 g of concentrate with 50% of bone, 11·6 g of concentrate with 22% of bone and 945·9 g of various residues containing between 1·2 and *c.* 0·05% of bone. Details of the scaled-up procedures are given below, while the results are presented pictorially in text-fig. 2.

Scaled-up Procedure C (Experiment 34)

This was employed only once, as follows: 1000 g of well-washed 0·7–0·85 mm residue from 31·9 kg of the Cliff End Bone Bed was divided into two equal portions, each being placed in a 1 litre 'Pyrex' beaker. Domestic paraffin, 300 ml, and cold tap-water, 250 ml, were added to one of the beakers, and gently agitated; after settling, the water/paraffin mixture was decanted through a 0·5 mm sieve into the other beaker. This decantation process from one beaker to the other was repeated for a total of 500 times over a period of 100 minutes. During each of these 500 extractions, the interface between the paraffin and the water held a small quantity of material slightly enriched in bone, which accumulated on the sieve as 239·8 g of concentrate. For further details see text-fig. 2.

Scaled-up Procedure A (Experiment 35)

This too was employed only once, as follows. The 758·4 g of the tailings from Experiment 34 was placed in a square tin of side 23 cm and height 10 cm, along with 76 ml of tetrachloroethylene and 760 ml of a 1% solution of 'Fairy Liquid' in cold tap-water. Small pieces of foamed polystyrene sheet (cut from 'takeaway'

hamburger boxes) were added, and the mixture agitated gently. Pieces of the polystyrene were removed as and when they had acquired a coating of material enriched in bone, and were replaced with fresh polystyrene. This process was continued until the increasingly poorer yield and lengthening extraction times made further extraction unprofitable; in total it took 96 minutes and used 225 pieces of polystyrene with an estimated surface area of 9000 cm^2.

After removal from the tin, each piece of polystyrene was rinsed in cold water to remove non-adherent material not significantly enriched in bone, and was then dissolved in chloroform to allow the recovery of the concentrate. In total, 133·4 g of concentrate, containing 5·9% by weight of bone, was recovered (for further details see text-fig. 2).

Scaled-up Procedure B

The excellent results obtained with this procedure rapidly made it the 'workhorse' of this exercise, and it was used for all four of the remaining experiments. The most impressive of these (Experiment 38) is described below.

The 622·5 g of the tailings from Experiment 35 was placed back in the square tin used earlier, and wetted with 12·4 ml of domestic paraffin and enough 1% 'Fairy Liquid' solution to cover it to a depth of *c.* 5 mm (311 ml). Paraffin wax, recovered from earlier experiments and weighing 213 g, was melted in hot water and cast into thin flakes by pouring on to aluminium foil. One-fifth of these flakes were added to the tin, and the mixture gently agitated. Material enriched in bone slowly accumulated on the floating paraffin wax flakes, which were removed from the tin after 12 minutes agitation. Another four such extractions were then performed using the rest of the paraffin wax flakes, and with a fresh 12·4 ml of domestic paraffin being added to the tin before each extraction. When these had been completed, the paraffin wax was melted in hot water (containing a little detergent) to recover both the concentrate, which sank to the bottom of the hot water, and the paraffin wax, which floated on the surface. The molten paraffin wax was cast into flakes on aluminium foil and used in the next five extractions.

In all, twenty-five extractions were carried out as described above, the paraffin wax being recovered and recycled after every five extractions. The aqueous phase in the tin was replenished as necessary to compensate for losses. Similarly, fresh 12·4 ml aliquots of paraffin were added before each of extractions 1–11, 16, and 21, the spacing of the additions being as judged necessary to maintain both the quality and the quantity of the concentrates at a satisfactory level.

The first twenty extractions yielded 15·9 g of concentrate containing 15% of bone; as the starting material of the experiment contained only 0·5% of bone, this represents an enrichment of about thirtyfold. Other results from the experiment are shown in text-fig. 2.

In addition to its excellent performance, the scaled-up Procedure B described offers considerable economic advantages, due in large measure to the ease with which its most expensive component, the paraffin wax, can be recycled for reuse. As an example may be cited the paraffin wax used in the above experiment, which was originally obtained by melting domestic candles and which then weighed 383 g. It was used in all four of the scaled-up Procedure B experiments of text-fig. 2 and in another two experiments (not reported), during the course of which it was recycled twenty-two times. After this, it weighed 135 g and was still of suitable quality to be used again, if this had been necessary. Indeed, if anything, its effectiveness as a 'substrate' material had been improved by its being softened by the paraffin it had imbibed, which facilitated the adhesion of bone particles.

CLOSING REMARKS

How does the Interfacial Method stand at present? Even in its present embryonic form, the Interfacial Method is exceptionally useful in the recovery of microvertebrate fossils from sediment residues. It is effective over a wide range of gangue types and particle sizes, and with sediment residues containing both high and low concentrations of fossil bone. As a result, by multi-stage extractions one should be able to obtain high-grade bone concentrates from a wide range of poorly fossiliferous starting materials. The various embodiments of the Interfacial Method are all of very low operating cost, both in terms of money and manpower. Whilst subject to a variety of poorly understood variables, the Interfacial Method seems basically to be resilient in this regard, very few of the experiments that have been performed being total failures in the sense that no separation of bone from gangue minerals occurred at all. As further evidence of this fundamental resilience, the three embodiments described in this account (Procedures A, B, and C) use widely different materials and working procedures.

How could the Interfacial Method be further developed? The most exciting development would be a machine for the large-scale separation of bone from sediment residues; this should be possible by mechanization of Procedure C, whose simplicity and low cost makes it ideal for the purpose. Apart from this, the work described in this account needs to be consolidated, and in particular, the applicability of Procedures B and C to a comprehensive range of gangue types and particle sizes awaits study. Although residues finer than 0·35 mm have not yet been investigated, I anticipate that the Interfacial Method will prove especially useful in the recovery of conodonts and probably of phosphatized invertebrate microfossils. Other working procedures and 'solvent'/'substrate' systems also need to be investigated, for example, could paint films be useful 'substrates' with certain 'solvent' systems? The possibilities are endless.

On a more speculative level, perhaps the phenomenon on which the Interfacial Method is founded occurs in nature, and has played a part in the formation of certain kinds of bone bed, particularly those associated with bituminous sediments.

What will be the significance of the Interfacial Method for vertebrate palaeontology? Even now the Interfacial Method should enable comprehensive microvertebrate faunas to be obtained from sediments where they are at present either unknown, or are in terms of time and money, largely inaccessible to the collector. This in its turn should lead to an increased knowledge of certain obscure groups, notably Mesozoic mammals, and give a stimulus to the growing field of vertebrate palaeoecology by allowing faunal lists to be more frequently established on a quantitative basis.

But perhaps the single most important consequence of the Interfacial Method will be sociological. Up to now, microvertebrate fossils have been collected mainly by professional workers based in Europe and North America, who have been the only ones with the financial and manpower resources necessary when using earlier methods. The Interfacial Method will not only greatly assist these workers, but hopefully will also lead to a large increase in the number of active collectors, notably in countries where the resources available for palaeontology are small. It is also ideally suited to the amateur collector. This increase in manpower should lead to a corresponding increase in the number of productive localities and in their stratigraphic and geographic distribution. This, of course, will greatly enlarge the amount of fossil material available to the palaeontologist. When fully developed and widely applied, the Interfacial Method should have a highly stimulating effect upon vertebrate palaeontology.

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